SELECTIVE INHIBITION OF TRANSLATION IN TRANSFORMED CELLS

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1. Introduction

Cancer chemotherapy is based mainly on agents that, although cell-cycle specific inhibitors, do not normally show a selective effect on transformed, as opposed to normal-growing, cells [1]. Under special conditions, however, some compounds act selectively on transformed cells. For instance, compounds that arrest normal cells in the G1 phase of the cell-cycle combined with S phase inhibitors selectively kill transformed proliferating cells [2–6]. Unfortunately, many of these selective methods use agents too toxic for cancer therapy.

Several procedures are now available to reversibly permeabilize mammalian cells to low molecular weight compounds [7,8]. One of these methods, which uses ATP combined with a saline buffer, specifically permeabilizes transformed cells [7]. However, both normal and transformed cells exposed to the permeabilizing conditions are equally viable when replaced in normal culture medium. We reasoned that this selective permeabilization could provide a method for specifically killing transformed cells with inhibitors to which normal or transformed untreated cells are normally impermeable. An inhibitor of this kind is hygromycin B, an aminoglycoside antibiotic produced by *Streptomyces hygroscopicus*. Although this compound powerfully blocks protein synthesis in cell-free systems [9], it is ineffective in intact mammalian cells, owing to failure to penetrate the plasma membrane [10]. We have shown that this antibiotic readily inhibits translation in virus-infected cells, as these cells have modified permeability to low molecular weight compounds [10,11].

2. Materials and methods

Mouse fibroblasts Balb 3T3 and Kirsten sarcoma virus-transformed 3T3 cells (K-A31) were kindly donated by Drs M. Barbacid and S. Aaronson. They were propagated in Falcon flasks containing Dulbecco's modified Eagle's medium supplemented with 10% newborn calf serum (Gibco).

The permeabilization of cells was carried out basically as in [7]. For this purpose, 0.5 mM permeabilization buffer (100 mM Tris–HCl (pH 8.2), 50 mM NaCl, 50 μM $CaCl_2$, 5 mg dextran/ml, 500 and 0.5 mM ATP) was added to cells cultured in 24-well dishes. The permeabilization was stopped by adding fresh culture medium supplemented with 10% newborn calf serum.

Protein synthesis was estimated by placing the cells in methionine-free culture medium supplemented with 0.5% newborn calf serum. [^{35}S]Methionine (1000 Ci/mmol; 250 000 cpm/well) 10 μl, was added and the cells were finally processed by precipitating the cell monolayer with 5% trichloroacetic acid, followed by 3 washes with ethanol. The cell precipitate was dried under an infrared lamp, dissolved in 0.3 ml 0.1 N NaOH and radioactivity in 0.150 ml measured in an scintillation spectrophotometer.

3. Results and discussion

Balb 3T3 and Kirsten sarcoma virus-transformed Balb 3T3 cells (K-A31) were a placed in permeabilization medium plus ATP in the presence of 0.5 mM hygromycin B for different times. After this treatment the cells were washed with fresh culture medium and protein synthesis was measured. Figure 1 shows that 5 min treatment was sufficient to block protein synthesis almost completely in transformed K-A31 cells, whereas a parallel treatment did not affect translation in normal 3T3 cells. Furthermore, 1 day after this treatment massive mortality of trans-

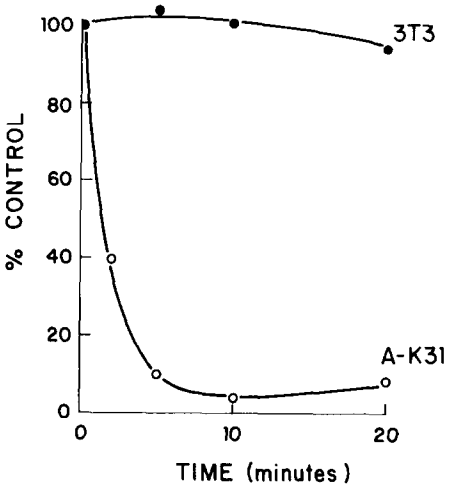

Fig.1. Balb 3T3 and K-A31 cells were cultured in Linbro dishes (24 wells) in Dulbecco's modified Eagle's medium containing 10% calf serum. The cells were permeabilized with 0.5 ml medium P containing: 100 mM Tris–HCl (pH 8.2), 50 mM NaCl, 50 μM $CaCl_2$, 5 mg dextran/ml, 500 and 0.5 mM ATP, and 0.5 mM hygromycin B. After treatment at 37°C for the indicated time 1 ml medium containing 10% calf serum was added and 1 h later, the medium was replaced by 0.5 ml methionine-free medium containing 0.5% calf serum and 10 μl [^{35}S]methionine (1000 Ci/mmol; 250 000 cpm/well) and the cells were incubated for another 1 h at 37°C. Finally, the radioactivity incorporated into protein was determined as in [10].

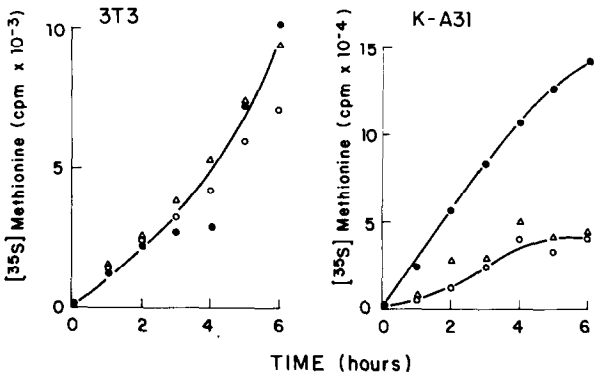

Fig.2. Cells, grown as indicated under fig.1, were permeabilized with 0.5 ml medium P, plus 0.1 mM ATP, plus the indicated amount of hygromycin B. After 10 min at 37°C, the cells were labelled with [^{35}S]methionine as indicated above. (●—●). Control; (△—△) 5 X 10^{-4} M hygromycin B; (○—○) 10^{-3} M hygromycin B.

formed cells was observed and, another day later, virtually all transformed cells permeabilized in the presence of hygromycin B were dead. However, 3T3 cells continued synthesising proteins at control levels.

Several experiments were carried out to test the effects of different inhibitors, of ATP and of the time when hygromycin B was added. Hygromycin B at $\geqslant$ 0.5 mM was sufficient to kill transformed cells if the antibiotic was present for 5–10 min during permeabilization. Moreover, ATP was not an absolute requirement to permeabilize transformed K-A31 cells: 40% inhibition by hygromycin B was obtained in its absence, but 0.1–0.5 mM ATP raised the inhibition to 90%. On the other hand, addition of the antibiotic with Eagle's serum-free medium after the permeabilization period had no effect on protein synthesis by K-A31 cells, indicating that the membrane seals rapidly after the addition of fresh culture medium.

The time course of protein synthesis both in 3T3 and K-A31 cells permeabilized in the absence or presence of various concentrations of hygromycin B is shown in fig.2. Transformed cells treated with 1 mM hygromycin B during the permeabilization period completely stopped the synthesis of new proteins 4 h after treatment, whereas 3T3 cells continued translation almost at control levels.

We also tested for the entry of high molecular weight inhibitors into transformed cells and found that in the presence of 40 μg α-sarcin/ml [12] even 60 min incubation in permeabilizing medium plus ATP, failed to block translation in K-A31 cells. This treatment then, would not seem to render K-A31 cells permeable to proteins.

The use of this permeabilisation method opens up the possibility of finding a number of selective inhibitors for transformed cells. The common requirement for these inhibitors is impermeability to cells under normal conditions. Unfortunately, most, if not all, anticancer drug screening procedures, discard this kind of drug. Although the therapeutical potential of this technique is still far from clear, because of the conditions used to permeabilize transformed cells, we believe it should be possible to find a mild non-toxic procedure to permeabilize cancerous cells specifically. Such an achievement would provide the rationale for a new approach to cancer therapy.

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References

[1] De Vita, V. T. and Busch, H. eds (1979) Cancer Drug Development: pt A. Methods in Cancer Research, vol. 16, Academic Press, New York.
[2] Pardee, A. B. and James, L. (1975) Proc. Natl. Acad. Sci. USA 72, 4994–4998.
[3] O'Neill, F. J. O. (1975) Cancer Res. 35, 1311–1315.
[4] Rozengurt, E. and Po, C. C. (1976) Nature 261, 701–702.
[5] Bradley, M. O., Kohn, K. W. and Sharkey, N. A. (1977) Cancer Res. 37, 2126–2131.
[6] Warrington, R. C. (1978) J. Natl. Cancer Inst. 61, 69–73.
[7] Rozengurt, E., Heppel, L. A. and Friedberg, I. (1977) J. Biol. Chem. 252, 4584–4590.
[8] Castellot, J. J., Miller, M. R. and Pardee, A. B. (1978) Proc. Natl. Acad. Sci. USA 75, 351–355.
[9] Gonzalez, A., Jimenez, A., Vázquez, D., Davies, J. E. and Schindler, D. (1978) Biochim. Biophys. Acta 521, 459–469.
[10] Contreras, A. and Carrasco, L. J. (1979) Virology 29, 114–122.
[11] Carrasco, L. (1978) Nature 272, 694–699.
[12] Vázquez, D. (1979) Inhibitors of Protein Biosynthesis. Molecular Biology, Biochemistry and Biophysics (Kleinzelle, A. et al.) vol. 30, Springer-Verlag, Berlin.